IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	Neil Andrew Williams, *et al.*
Application No. 09/600,060	Filing Date: July 10, 2000
Title of Application:	Agent for Treating Allergic or Hypersensitivity Condition
Confirmation No. 6761	Art Unit: 1644
Examiner	Phuong Huynh

Declaration Pursuant To 37 C.F.R. § 1.132

I, Professor. Neil Andrew Williams, hereby declare and state as follows:

1. I have a Bachelor of Science (1985) from the University of Birmingham, Birmingham, England, and a PhD: (1989) from the University of Bristol, in Bristol England. I am currently employed as a Professor of Immunology and Head of Department at the Dept. of Cellular & Molecular Medicine, University of Bristol, School of Medical Sciences. I have over 17 years of experience as a researcher in the field of immunology. I am a named co-inventor of a group of issued patents and named author or co-author of over 80 technical papers. My curriculum vitae is attached as Exhibit A.

2. I am the same Professor. Neil Andrew Williams named as the co-inventor for the above identified application Serial No. 09/600,060 (" '060 application") and I am aware of the contents of the application describing methods and agents for treating a number of diffrent conditions.

3. I am aware that The United States Patent & Trademark Office has indicated that the '060 application lacks a disclosure of the claimed invention that would enable a person of ordinary skill in the art of immunology to practice the invention. It is my opinion that the patent specification teaches the use of an effective amount of Ctx, Etx,

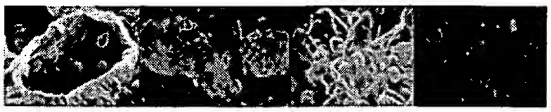
CtxB, and EtxB for co-administration with an allergen/antigen and would not require undue experimentation.

4. However, in order to provide additional information in the patent application file, I directed a study which was conducted by me and students acting under my supervision. The report of the study is attached as Exhibit B and I hereby incorporate the contents of the report into this declaration. The study and the report specifically disclose an effective amount of EtxB used in mucosal administration to prevent or treat delayed type hypersensitivity (a Type IV allergy), specifically, hypersensitivity to Hemocyanin, Keyhole Limpet (KLH) and to Chicken Egg White Albumin (OVA). This testing model is useful to analyze Type IV allergies which include reactions such as contact dermatitis as from exposure to poison ivy. In the study, EtxB was used as a therapeutic agent for intranasal administration in mice, and the study provides a basis for determination of an effective amount of EtxB used in mucosal administration humans and other mammals, and further provides sufficient information to enable mucosal administration of Ctx, Etx, and CtxB in addition to EtxB. The study shows that the use of EtxB pretreatment significantly reduced the level of delayed-type hypersensitivity (DTH) observed for both antigens tested. These data show that EtxB can be used as an effective means of preventing a type IV allergic condition, and that the ability of EtxB to inhibit an allergic response is not restricted by the nature of the sensitising antigen

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on my information and belief are believed to be true; further that the statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of my application or any patent issued thereon.

Date: 30th January, 2006 By: Neil Andrew Williams

University of BRISTOL **Cellular & Molecular Medicine**



Staff
Prof N Williams
Research
Postgraduate Study
Undergraduate Admissions
Seminars
Vacancies
About the Department
Contacts
News

Academic Staff

Professor Neil A. Williams

B.Sc. (1985) University of Birmingham
PhD: (1989) University of Bristol

PhD Projects

Professor of Immunology

Contact Details
Dept. of Cellular & Molecular Medicine, University of Bristol,
School of Medical Sciences, Bristol, BS8 1TD
Tel: +44 (0)117 928 7886 (internal 7886)
Fax: +44 (0)117 928 7896
Email: neil.a.williams@bristol.ac.uk

Past Research

- The cell biology of epidermal Langerhans cells; antigen presentation and role in epidermal infection.
- The pathogenesis of herpes simplex virus infection.

Current Research

- Research ongoing within the Williams laboratory focuses on basic and applied aspects of immunity at mucosal surfaces. Projects are aimed at defining the unique way in which antigen is taken up and presented to cell of the immune system at mucosal surfaces. In the context of most non-replicating protein antigens, the responses which result from mucosal antigen delivery involve specific down regulation of immune reactivity (tolerance). Work is focused on understanding the mechanisms by which tolerance is induced and maintained. Further work seeks to determine how certain proteins can specifically modulate the immune system following mucosal delivery and trigger immunity rather than tolerance. Cholera toxin (Ctx) and its relative, *Escherichia coli* heat-labile enterotoxin (Etx), act in this way and can be used as highly effective mucosal adjuvants triggering strong protective immunity to unrelated antigens, while other antigens, and their B-subunits (CtxB and EtxB respectively) can be used to prevent or treat autoimmune diseases. We are investigating these molecules as both adjuvants (for herpes simplex virus vaccination) and immunotherapeutics (for treating autoimmunity and transplant rejection). In addition, we are currently defining at the cellular and cell signaling level the precise processes by which these molecules modulate cells of the immune system.

Present co-workers

- Dr Claire Richards (post-doctoral research fellow; The Wellcome Trust): Mucosal vaccines against herpes simplex virus
- Dr Kwok-Keung Tong (post-doctoral research associate; Arthritis Research Campaign): Modulation of arthritis by *E. coli* heat-labile enterotoxin B-subunit
- Mrs Rachel Williams (research technician; Edward Jenner Institute for Vaccine Research): Biology of CD8 T cell apoptosis

Meningococcal Research Group (joint with Dr Robert Heyderman)

- Dr Victoria Davenport (post-doctoral research associate; Meningitis Research Foundation): Mucosal immunity to Group B *Neisseria meningitidis*
- Mrs Rachel Horton (post-graduate student; Public Health Laboratory Service): Mucosal immunity to Group B *Neisseria meningitidis*

Cancer Vaccines (joint with Dr David Morgan)

- Dr Rhiannon Jenkinson (post-doctoral research associate; Cancer UK): The role of tumours in the induction of peripheral tolerance

Recent Publications

Journal papers

Plant, A. & **Williams, N.A.** (2004) Modulation of the immune response by the cholera-like enterotoxins. *Curr. Topics Med. Chem.* 5, 509-519.

Apostolaki, M. & **Williams, N.A.** (2004) Nasal delivery of antigen with the B-subunit of *Escherichia coli* heat-labile enterotoxin augments antigen-specific T cell clonal expansion and differentiation. *Infect. Imm.* 72, 4072-4080.

Plant, A., Williams, R.M., Jackson, M.E. & **Williams, N.A.** (2003) The B-subunit of *Escherichia coli* heat labile enterotoxin abrogates oral tolerance, promoting predominantly Th2 type responses. *Eur. J. Immunol.* **33**, 3186-3195.

Davenport, V., Guthrie, T., Finlow, J., Borrow, R., **Williams, N.A.** & Heyderman, R.S. (2003) Evidence for naturally acquired T cell mediated mucosal immunity to *Neisseria meningitidis*. *J. Immunol.* **171**, 4263-4270.

Smartt, H.J.M., Elder, D.J.E., Hicks, D.J., **Williams, N.A.** & Paraskeva, C. (2003) Increased NF-κB DNA binding but not transcriptional activity during apoptosis induced by the COX-2-selective inhibitor NS-398 in colorectal carcinoma cells. *Brit. J. Cancer* **89**, 1358-1365.

Jordan, R.W., Hamilton, T.D.C., Hayes, C.M., Patel, D., Jones, P.H., Roe, J.M. & **Williams, N.A.** (2003) Modulation of the humoral immune response of swine and mice mediated by toxigenic *Pasteurella multocida*. *FEMS Immunol. Microbiol.* **39**, 51-59.

Richards, C.M., Case, R., Hirst, T.R., Hill, T.J. & **Williams, N.A.** (2003) Protection against recurrent ocular HSV-1 disease following therapeutic vaccination of latently infected mice. *J. Virol.* **77**, 6692-6699.

Salmond, R.J., Hirst, T.R. & **Williams, N.A.** (2003) Selective induction of CD8+CD4- thymocyte apoptosis mediated by the B-subunit of *Escherichia coli* heat-labile enterotoxin. *Imm. Lett.* **88**, 43-46.

Fraser, S.A., deHaan, L., Hearn, A.R., Bone, H.K., Salmond, R.J., Rivett, A.J., **Williams, N.A.** & Hirst, T.R. (2003) Mutant *Escherichia coli* heat-labile toxin B-subunit that separates toxoid-mediated signalling and immunomodulatory action from trafficking and delivery functions. *Inf. Imm.* **71**, 1527-1537.

Salmond, R. Luross, J.A. & **Williams, N.A.** (2002) Immune modulation by the cholera-like enterotoxins. *Expert Reviews in Molecular Medicine*. 1 October, http://www.expertreviews.org/02005057h.htm.

Turcanu, V. Hirst, T.R. & **Williams, N.A.** (2002) Modulation of human monocytes by *Escherichia coli* heat-labile enterotoxin B-subunit; altered cytokine production and its functional consequences. *Immunol.* **106**, 316-325.

Bone, H.K., Eckholdt, S. & **Williams, N.A.** (2002) Modulation of B lymphocyte signalling by the B-subunit of *Escherichia coli* heat-labile enterotoxin. *Int. Immunol.* **14**, 647-658.

Luross, J.A., Heaton, C.P.E., Hirst, T.R., Day, M.J. & **Williams, N.A.** (2002) *Escherichia coli* heat-labile enterotoxin B-subunit prevents autoimmune arthritis through the induction of regulatory $CD4^+$ T cells. *Arthritis Rheum.* **46**, 1671-1682.

Salmond, R., Pitman, R.S., Jimi, E., Soriani, M., Hirst, T.R., Ghosh, S., Rincon, M. & **Williams, N.A.** (2002) $CD8^+$ T cell apoptosis induced by *Escherichia coli* heat-labile enterotoxin B subunit occurs via a novel pathway involving NF-κB-dependent caspase activation *Eur. J. Immunol.* **37**, 1737-1747.

Nicholls, S.M. & **Williams, N.A.** (2001) MHC matching and mechanisms of alloactivation in corneal transplantation. *Transplant.* **72**, 1491-1497.

Soriani, M., **Williams, N.A.** & Hirst, T.R. (2001) *Escherichia coli* enterotoxin B subunit triggers apoptosis of $CD8^+$ T cells by activating

transcription factor c-Myc. *Infect. Immun.* **69**, 4923-4930.

Aman, A.T., Fraser, S., Merritt, E.A., Rodigherio, C, Kenny, M., Ahn, M., Hol, W.G.J., **Williams, N.A.**, Lencer, W.I. & Hirst, T.R. (2001) A mutant cholera toxin B subunit that binds GM1-ganglioside but lacks immunomodulatory or toxic activity. *Proc. Natl. Acad. Sci. USA.* **98**, 8536-8541.

Bone, H.K. & **Williams, N.A.** (2001) Antigen-receptor cross-linking and lipopolysaccharide trigger distinct phosphoinositide 3-kinase-dependent pathways to NF-κB activation in primary B cells. *Int. Immunol.* **13**, 807-816.

Turcanu, V. & **Williams, N.A.**. (2001) Cell identification and isolation on the basis of cytokine secretion: a novel tool for investigating immune responses. *Nature Med.* **7**, 373-376.

Richards, C.M., Aman, T., Hirst, T.R., Hill, T.J. & **Williams, N.A.** (2001) Protective mucosal immunity to ocular herpes simplex virus type-1 infection in mice using *Escherichia coli* heat-labile enterotoxin B-subunit as an adjuvant. *J. Virol.* **75**, 1664-1671.

Reviews

Williams, N.A., Hirst, T.R. & Nashar, T.O. (1999) Immune modulation by the cholera-like enterotoxins: from adjuvant to immunotherapeutic. *Immunol. Today* **20**, 95-101.

Hirst, T.R., Nashar, T.O., Pitman, R.S. & **Williams, N.A.** (1998) Cholera and related enterotoxins as potent immune modulators. *J. Applied Microbiol.* **84**, 26S-34S.

Nashar, T.O., **Williams, N.A.**, & Hirst, T.R. (1998) Importance of receptor binding in the immunogenicity, adjuvanticity and therapeutic properties of cholera toxin and *E. coli* heat-labile enterotoxin. *Med. Microbiol. Immunol.* **187**, 3-10.

Hirst, T.R., Nashar, T.O., Miller, D.G. & **Williams, N.A.** (1998) Immunomodulatory properties of cholera toxin and related enterotoxins. In: *Recent Advances in the pathogenesis of gastrointestinal bacterial infections*, EAGE Postgraduate Course, Nice. Eds: Rampal, P. & Boquet, P. pp165-175.